bio-protocol Preprint

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Protein expression for crystal generation

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An abbreviated version of this protocol was published in eLIFE in Feb 2016

Estrogen receptor alpha somatic mutations Y537S and D538G confer breast cancer endocrine resistance by stabilizing the activating function-2 binding conformation

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Detailed protocol

Updated Expression and Purificaiton Protocol

Running/Lysis Buffer 1L keep refrigerated.

- 50 mM HEPES pH 8 (Tris pH 8 is fine too)
- 500 mM NaCl
- 0.5 mM TCEP
- 20 mM Imidazole
- Fill with DI H2O to 900 mL
- Add 100 mL glycerol while stirring.

Elution Buffer 500 mL

- 50 mM HEPES pH 8
- 1 M NaCl
- 500 mM TCEP
- 500 mM Imidazole
- Fill with DI H2O to 450 mL
- Add 50 mL glycerol (under sink) while stirring.

Resuspension

- 10 mL cold running buffer per gram cell paste.
- 1 EDTA-Free pic tab per 50 mL lysis (Pierce Cat#A32965).
- Thoroughly resuspend on ice by pipetting.

Lysis with Sonication

- Per 50 mL lysate, 5 s on/off cycles for 5 minutes of total sonicating at 80% power on ice. Sonicator used is a Fisherbrand Model 120 Sonic Dismembrator with a horn suitable for 50 mL sonication volumes.

Clarifying Lysate

- Centrifuge at >18,000 xg for 30 minutes at 4^{o}C using the fixed angle rotor. Lysate should be clear and yellow.
- Transfer to a new tube to separate soluble and insoluble fractions, make sure that no insoluble debris transfers over.

Purification

- Purification was performed using a BioRad NGC Quest FPLC with a sample pump.
- We use a BioRad Nuvia 5 mL IMAC column for affinity purification. FPLC should have the running/lysis buffer loaded on pump A and the elution buffer on pump B.
- Clean the column by flushing 3 CV of 100% pump B.
- Equilibrate the column with 5 CV of pump A or until UV baseline is reached.
- Place sample pump line into the clarified lysate and pull 5 mL through the pump to prime it and purge any air.
- Load the sample directly onto the column, wash with 100 pump A until baseline is reached, do a linear gradient elution from 0 to 100% pump B.
- Pick peak fractions and run 20 uL (with 20 uL load dye) on a SDS-PAGE gel along with ladder.
- Combine fractions with the correct band corresponding to protein of interest take a reading on the nanodrop. A260/280 should be <0.8. If greater, add DNAse. Use extinction coefficient to determine molarity.

TEV Cleavage and Dialysis

- Add 1:5,000 HisTev, place in dialysis membrane, and dialyze against 4 L of running buffer overnight at 4^{o}C with stirring.

Reverse Ni-NTA

- Load dialyzed protein onto the IMAC column as before, collect flow through. Run 20 mL running buffer over column and collect. In a separate fraction, run 100% pump B/elution buffer to elute the cleaved His tag and His-TEV.
- While this is running make SEC buffer, which is running buffer without imidazole. Degas for at least 30 minutes with stirring. Once reverse Ni-NTA protocol is complete, start equilibrating the SEC column at 1 mL/min for at least 1 CV.

Gel Filtration

- Using a spin concentrator with a 30 kDa molecular weight cutoff, concentrate the protein to at least 5 mg/mL using the filtration concentrators. Use the swinging bucket rotor at 4^{o}C, 4,000xg for 5-15 minutes at a time. Concentrated to no less than 1 mL, ideally to 5 mL. Protein should not be concentrated to more than 20 mg/mL, make sure you are taking nanodrop readings throughout the concentrating. Ideally, the A260/280 ratio will decrease as the A280 increases. Remember that the absorbance isn't always 1:1 with the A280 given the extinction coefficient of the protein, but in general you need an A280 of at least 3 to see fractions on the SEC.
- Run fractions on the gel. ER LBD typically elutes around 30-60 kDa on the SEC. Concentrate to 5-20 mg/mL and flash freeze for later use. If time, set up crystal trays right away.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Fanning, S. W.(2020). Protein expression for crystal generation. Bio-protocol Preprint. bio-protocol.org/prep538.
2. Fanning, S. W., Mayne, C. G., Dharmarajan, V., Carlson, K. E., Martin, T. A., Novick, S. J., Toy, W., Green, B., Panchamukhi, S., Katzenellenbogen, B. S., Tajkhorshid, E., Griffin, P. R., Shen, Y., Chandarlapaty, S., Katzenellenbogen, J. A. and Greene, G. L.(2016). Estrogen receptor alpha somatic mutations Y537S and D538G confer breast cancer endocrine resistance by stabilizing the activating function-2 binding conformation. eLIFE. DOI: 10.7554/eLife.12792